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This report discusses (1) the somatic-mutation theory of cancer, (2) the mutagenicity of carcinogenic compounds and (3) the quantitative correlation between mutagenicity and carcinogenicity. The Committee on Chemical Environmental Mutagens concluded that further research and analysis of data are necessary as a basis for determining whether short-term mutagenicity tests possibly can be used for quantitative prediction of human carcinogenicity.

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A Feasibility Study

Committee on Chemical Environmental Mutagens

Board on Toxicology and Environmental Health Hazards

Commission on Life Sciences

National Research Council

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Board on Toxicology and Environmental Health Hazards
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PREFACE

The Committee on Chemical Environmental Mutagens (CCEM) has been asked to determine whether it is feasible at this time to study the relationship between mutagenic and carcinogenic potencies. The problem of studying this relationship begins with the definitions of mutagenic and carcinogenic potencies. Often, mutagenic potency must be described according to the biologic test in which it appears. It is usually defined as the amount of increase in the response of a test organism or test system at a particular concentration of the chemical under test. Potency has been variously expressed as other measures that are equivalent or closely related, including the initial slope of the dose-response curve, the lowest effective dose, the doubling dose, the mutation effect at 50% cell death, and the radiation-equivalent dose.[70] The definition of carcinogenic potency likewise depends on the biologic system that generates the data. A recent definition of carcinogenic potency is the dose of a chemical carcinogen required to produce tumors in half the tumor-free test animals in a normal lifetime.

Several issues arise in the calculation of mutagenic or carcinogenic potency. We need to know whether the data are accurate and whether the results can be extrapolated among different experimental organisms. The success of past attempts to make comparisons can be used to suggest the likely outcome of future efforts that use the same approaches. And, it is important to examine the rationale of any relationship between the processes of carcinogenesis and mutagenesis.

It is very important to determine whether algorithms can be created for extrapolating short-term test data to animal models and, ultimately, to human carcinogenesis. Without this, human risk assessment cannot be approached quantitatively through these types of toxicity tests. This report will discuss the critical factors necessary to determine if there is a quantitative correlation between mutagenesis and carcinogenesis. After assessing the factors, the Committee offers general recommendations for how such a quantitative study might be done.

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THE SOMATIC-MUTATION THEORY OF CANCER

CHROMOSOMAL ABERRATION

In 1914, Boveri[26] proposed a link between mutagenesis and carcinogenesis. But the molecular biology of cancer is still elusive, and, although mutation is thought to be intimately connected with cancer, its exact role in the disease cannot be definitively described. Recent reviews have highlighted the uncertainty.[32-34,51,67,105,114]

Boveri's hypothesis stemmed from his observations on chromosomal aberrations in tumor cells. Chromosomal rearrangements continue to be found in some cancers, but no general pattern has emerged. Klein[61] has discussed the apparent tissue-specific involvement of different chromosomes in tumor-associated nonrandom karyotype changes. For example, human B-cell lymphoma is associated with the $14q^{+}$ band, Burkitt's lymphoma with a reciprocal 8:14 translocation, and myeloid differentiation with changes in chromosomes 22 and 9. Wiener *et al.*[119] have described a trisomy-15 in murine B-cell lymphoma that suggests a gene duplication on chromosome 15, and the chromosomal instability associated with Bloom's syndrome is thought to predispose those afflicted to leukemia and other cancers.[110] Other examples of chromosomal aberrations associated with neoplasia include a chromosome 3:8 translocation with hereditary renal cell carcinoma,[40] a chromosome 13 deletion with retinoblastoma,[64] and a deletion in chromosome 11 with Wilms' tumor. In a report from the National Wilms' Tumor Study, Breslow and Beckwith[29] suggested that the proportion of Wilms' tumors due to inherited mutation may be substantially smaller than previously estimated. None of the 20 familial cases had the features associated with genetic tumors. Although cytogenetic analyses were not performed in this large study, that many familial cases were unilateral and did not depend on the subjects' ages suggested that mechanisms other than mutation may be responsible for most cases of Wilms' tumor.

These findings led Klein,[62] Cairns,[34] and Nevers and Saedler[86] to suggest that chromosomal rearrangements may be a gross manifestation of a critical gene-dosage effect. By chromosomal rearrangement, a set of endogenous cancer genes may come under the influence of more active transcriptional control. Recently, insertion of viral promoters has been shown to cause transformation in cell cultures by enhanced transcription of viral or endogenous oncogenes.[36,91] Endogenous oncogenes have been mapped on specific human chromosomes,[95,115] and there is evidence that reciprocal translocations may bring these genes under different promotional control.[62] Thus, chromosomal rearrangements (observed by using cytogenetic techniques) and small-scale transposition events (that can be detected only genetically) may both produce the same effect.

MOST ULTIMATE CARCINOGENS ARE MUTAGENS

Many chemical carcinogens have been tested for mutagenicity in the last decade. The development of the Salmonella/microsome reverse-mutation assay by Ames and colleagues[71,73] signaled the beginning of large-scale efforts. In combination with results from other in vitro mutagenicity tests, the Salmonella/microsome assay detects almost all carcinogens with mutagenic properties. The Committee expressed great confidence in a small test battery for detecting chemicals with mutagenic properties.[84] The Committee also agreed with other estimates (e.g., Brusick[31]) that approximately 90% of currently known animal carcinogens are bacterial mutagens.

However, the causal relationship between mutation and cancer is not as direct as these statistics imply, and the Committee was reluctant to assert mechanistic links in its initial report.[84] One reason is that some confirmed animal carcinogens have no mutagenic activity or are mutagenic in only a minority of the systems in which they are tested. For example, benzene and arsenic compounds are not mutagenic in the Salmonella/microsome assay, but do display activity in some other short-term assays.[21] Phenobarbital, succinic anhydride, and dieldrin are carcinogens that are negative in most mutagenicity assays.[54] The mechanisms by which these compounds and some others, such as the steroid hormones (e.g., 17β-estradiol), cause cancer are not known. Rubin[105] has stressed the difficulty in ascribing mutational mechanisms to cancers caused by a variety of nongenetic agents.

Cairns[34] has proposed that transposons may constitute a major mechanism in the development of cancer and has urged examination of agents that increase the frequency of transpositions. Many of these agents would not be detected in conventional in vitro mutagenicity assays, especially if eukaryotic and bacterial transposons behaved differently.

Fahmy and Fahmy[45,46] have begun studies on the chemical induction of transposition with the white (w^+) eye-color gene complex of Drosophila. Stable and nonstable genetic features of the complex are used to assess germinal mutation and somatic gene expression that results from transposition of a duplicate regulatory locus in w^+. Preliminary evidence derived from the few carcinogens tested suggests that mutagenic carcinogens have an influence on supposed transpositions, but this phenomenon is unrelated to a carcinogen's mutagenic potency. Further work is required to confirm and extend these findings. Eeken and Sobels[44] have studied chemically induced transposition at the singed bristle (sn) locus of Drosophila. Powerful mutagens, such as ethyl nitrosourea and methyl methanesulfonate, did not greatly influence reversion of insertion mutation, but DNA-repair defects (measured in the repair-deficient strain mei-9/mei-41) did interfere.

Although not all mutagenicity test data are in concordance with carcinogenicity data, most investigators believe that a relationship exists between mutagenesis and cancer. In addition to the high correlation between short-term mutagenicity and animal chemical-carcinogenicity data, there is experimental support for the multistage model of carcinogenesis. Recent reviews[63,65,80] have agreed that somatic mutation is likely to be the leading cause of initiation of the carcinogenic process.

Berenblum and Shubik[23] provided early evidence of a mutational mechanism in the initiation of cancer. Recently gathered evidence indicates that, in later stages of progression to malignancy (i.e., promotion), DNA may be unaffected.[22,69] However, as Cairns has pointed out,[33] promotional activity that produces cell proliferation can be accommodated in a multistage cancer model in which the first stage derives from somatic mutation. Other new data also reflect the mutational etiology of cancer. Weinberg et al.[116] and Barbacid et al.[101] have provided evidence that a single point mutation in the coding region of a human bladder proto-oncogene may be sufficient for its conversion to an oncogene that elicits a carcinoma. Although this finding awaits additional documentation,[7,81] it offers some support for mutation as an early part of the carcinogenic process.

ULTIMATE CARCINOGENS THAT ARE NOT MUTAGENS

From a regulatory standpoint, it is important to be aware of a set of potent human or animal carcinogens with no observed or only inconclusive mutagenic activity. Human exposure to many of these chemicals may be quite high. This phenomenon has created concern over the protection afforded by mutagenicity tests in predicting carcinogenic potential, because many of

these substances are present in the environment at high concentrations. Consequently, as Squire[112] emphasized, carcinogenicity testing in laboratory animals remains the primary basis for most regulatory decisions. Because no specific experimental factor accurately predicts human carcinogenicity, Squire proposed ranking potential carcinogens according to several factors, including genotoxicity tests.

There are two principal reasons why some chemical carcinogens are not detected in mutagenicity tests. The first is obvious: several chemicals (e.g., steroid hormones) either cause cancer by nongenotoxic means or alter DNA indirectly; these chemicals do not appear to be mutagens under any conditions. The second reason is that mutagenicity tests are inefficient because of inadequate permeability, insensitivity of genetic end points, metabolic inadequacy, or other factors. To counter weaknesses of individual tests, the phylogenetic range of test batteries is broad and the sources of metabolic activation are varied. Thus, the probability is low that, if a battery of short-term tests were used, a confirmed genotoxic carcinogen could be legitimately shown to be nonmutagenic.

GENETICS OF CANCER

Investigators have approached the genetic etiology of cancer in several ways. One approach is experimental. Another involves the study of the inheritance of cancer in human populations and the effects of genetic disturbances in select populations. A third combines epidemiologic and experimental approaches in in vitro experiments with cells from human tumors that are known to be or suspected of being inherited genetically. The outcome of such approaches will be a description of the inheritance of cancer and an understanding of the mechanism responsible.

Cancer occurs in a few genetic syndromes,[63,65] and it is not unreasonable to assume a genetic basis for predisposition to the disease. However, a more obvious hereditary pattern of human carcinogenesis has not been found. Perhaps the lack of such a pattern is due to the multiple stages of the disease, each of which might require the action of separate genes. Perhaps cancer results from a polygenic trait in many cases. In lieu of strict simple Mendelian inheritance, the human population appears to be heterogeneous in its susceptibility to neoplasia. "Susceptibility" may involve a genetically influenced late-in-life change (i.e., like graying of hair); in susceptible persons, this change occurs earlier in life than it does in less susceptible persons.

Studies of human cell transformation in vitro reflect efforts to understand the mutational basis of carcinogenesis. Barrett and colleagues[15,16] have shown that diethylstil-

bestrol (DES) induced neoplastic and morphologic transformation at a frequency similar to that of benzo[a]pyrene. However, DES failed to induce somatic mutation at two genetic loci, whereas benzo[a]pyrene was mutagenic. Thus, two independent mechanisms appeared to lead to the same effect. In a review of the literature, Parodi and Brambilla[87] claimed that transformation occurs 100-1,000 times more frequently than mutation at a defined concentration of chemical. Straus[114] asserted that the range of difference is one-tenth as great and pointed out the technical difficulties in comparing the two end points in divergent test systems. Using mammalian cells in culture, Huberman[57] studied mutagenesis at the ouabain locus and cell transformation and concluded that approximately 20 genes may be involved in one transformation event.

Cell hybridomas produced by cell fusion provide another in vitro system for examining mutationally induced transformation. Harris has discussed the heritability of malignancy on the basis of hybridoma data,[51] especially in the context of the phenomenon of chromosomal exclusion and tumorigenicity. He concluded that Mendelian segregation patterns of hybrid cells do not favor a truly dominant or recessive mode.

Harris's conclusion was supported by the more recent analysis of Sabin.[106] The suppression of malignancy by cell fusion and subsequent chromosomal loss also is stable and transmissible in some cases. However, the data are often inconsistent. For example, malignancy can be suppressed in naturally occurring human cancers by fusion of cancer cells to normal human fibroblasts, differentiating epithelial keratinocytes, and cancer cells of different somatic cell origin. But suppression has not yet been specifically linked either with a cancer cell of a particular origin or with a type of nonmalignant cell to which the cancer cell is fused. Attempts to correlate suppression with the segregation of specific chromosomes, even for tissue-specific neoplasia, have also been inconsistent. Although the retention of specific chromosomes and the suppression of the malignant phenotypes imply a dominant mechanism, in vitro data cannot yet properly distinguish a definitive segregation pattern.

Analysis of human populations indicates strongly that susceptibility to cancer varies among individuals and that genetic predisposition may be more important for the development of some tumors than others.[63] Moolgavkar and Knudson[80] argued that the development of hereditary tumors should not be overlooked on the grounds that they represent only a small fraction of all cancers; rather, they offer an opportunity to study the multistage process of carcinogenesis.

Mendelian analysis shows that almost all hereditary cancers segregate in an autosomal dominant fashion. Assuming the relationship to be strict, Moolgavkar and Knudson[80] commented that, "even though every cell in a susceptible tissue carries

the cancer gene, only a few of the cells go on to become malignant, which indicates that inheritance of the gene is not sufficient (at the cellular level) and that at least one other event is necessary for malignant transformation." In a two-stage cancer model, the other event is not necessarily mutation. Among many others, Clayson[37] has discussed non-genetic mechanisms for the second stage of a two-stage cancer model. Enhanced cellular proliferation has been identified as a possible participant in producing tumors.

Examples of autosomal dominant cancers are bilateral retinoblastoma, neuroblastoma, Wilms' tumor, and some carcinomas of the colon. McKusick[76] listed approximately 30 human cancers that may be inherited in a dominant fashion. These account for approximately 1% of all human cancers in the United States.[63] The total number of human cancer genes remains a matter of speculation; Moolgavkar and Knudson[80] suggested that there may be 100-200, whereas recent hybridization data between viral or human oncogenes and human DNA imply a range of only 10-20.[25] However, the sensitivity of nucleic acid hybridization techniques is probably not so great that all cancer genes can be detected definitively.

The even rarer recessively inherited cancers are important in understanding mechanisms. Two disorders, xeroderma pigmentosum (XP) and ataxia telangiectasia, occur in persons severely deficient in DNA repair; the deficiency probably increases their disposition to cancer. Persons with two other genetic syndromes, Fanconi's anemia and Bloom's syndrome, exhibit several abnormalities, including chromosomal gaps and DNA strand-breaking. By implication, these recessive disorders are linked mechanistically to increased cancer incidence. Again, however, the relationship demands scrutiny. Cairns[34] argued that XP patients have a greater frequency of cancer only of the skin, where UV-light-induced damage can occur. He asserted that exposure to other mutagens does not increase the incidence of other cancers beyond that in the general population. This anomaly cannot be easily explained, and a possible conclusion is that the cancers are not caused by the type of DNA damage that XP patients are unable to repair. However, the limited number of XP patients in these studies reduces the certainty of epidemiologic data. Indeed, some evidence suggests that an excess of cancers exists in XP patients in sites other than the skin.[66]

Garner and Hertzog[48] proposed the counterargument that XP patients should not, a priori, be expected to have a higher incidence of internal cancers. Organ-specific metabolism, DNA repair, and resistence to dimers and other lesions like those induced by UV may influence tumor formation.

Peto[92] has recently investigated the idea that there are different individual susceptibilities to cancer. From epidemiologic analyses, he set up three broad classes. The

first includes cases in which the increase in risk is more than a factor of 1,000. Cancers included in this group are bilateral retinoblastomas and the squamous cell skin cancers of XP patients. Although some persons are at tremendous risk, only a minute fraction of human cancers are in this category. The second category includes cancers for which a genetic predisposition leads to risks approximately 10-100 times higher than that in the general population. Peto argued that cancers in this group may account for a substantial proportion of all cancers, but their categorization by frequency may be very difficult to prove. He argued further that the relative risk in relatives of patients compared with the general population may be an order of magnitude less than the relative risk in suspectible persons compared with nonsusceptible persons. He gave three reasons for this: not all cancer patients are susceptible, even fewer relatives are susceptible, and the general population contains susceptible and nonsusceptible persons. Peto surmised that a third category is composed of persons whose genetic risk is increased by less than a factor of 10 and who may remain undetected in the population.

2

THE MUTAGENICITY OF CARCINOGENIC COMPOUNDS

THE SALMONELLA/MICROSOME TEST

The work of Ames and colleagues[1,71,73] has led to the development of microbial mutation assays to detect chemical carcinogens. Originally, analyses with the Salmonella/-microsome test were inductive; known animal carcinogens were tested for mutagenic activity for validation purposes. However, testing strategies have evolved, and short-term mutagenicity tests are now used to screen for chemicals with unknown carcinogenic properties. The Committee[84] has reviewed the large number of mutagenicity tests that constitute a test strategy. The organisms in these tests span the phylogenetic range from bacteria, such as salmonellae, to whole rodents. McCann and Ames[72] have argued that most carcinogens are mutagens and that mutagenicity screening tests would identify many chemical carcinogens without the need, in many cases, for expensive, lifetime rodent bioassays.

Evidence presented in this section suggests a qualitative relationship between the mutagenic and carcinogenic properties of most classes of chemicals. What has been challenged is the degree of concordance between the processes and what causes this qualitative correlation to break down. McCann[70] has discussed the use of short-term tests in relation to chemical carcinogenesis and cancer policy. She argued that short-term tests are valid experimental predictors of chemical carcinogenesis for four reasons: (1) although no test detects all carcinogens, most carcinogens are detected in a relatively few mutagenicity tests; (2) although some carcinogens are not detected in any test, a combination of tests increases the proportion of carcinogens that are detected; (3) positive results from the Salmonella/microsome test are usually confirmed by other tests, as are negative results and results with weakly active chemicals often confirmed; and (4) the percentage

of true noncarcinogens that are falsely positive in short-term tests is likely to be lower than validation studies suggest.

McCann and Ames[72] asserted that approximately 90% of chemical carcinogens would be mutagenic in the Salmonella/-microsome assay and that most noncarcinogens would not be mutagenic. Despite the need for more data to substantiate the extent of the correlation, it is generally accepted, and the Committee[84] concurs in the probable validity of these estimates.

In an exchange of views, Rinkus and Legator[102,103] and Ames and McCann[6] discussed the success rate of the Salmonella/microsome test in detecting carcinogens. Rinkus and Legator contended that about 77% of carcinogens that had been tested in the assay were detected as mutagens, according to data available in 1979, and Ames and McCann estimated this value as slightly higher, 82%. Most reviews--e.g., Purchase et al.,[99] Brusick,[31] Nagao et al.,[82] and this Committee[84] --have favored the claim that approximately 80-90% of carcinogens are mutagens, although such support is guarded by the perpetual call for more data.

Ames and Haroun[3] have also considered the criticisms of Lijinsky and colleagues[8-10] concerning the accuracy of the Salmonella/microsome test in detecting carcinogens. They reported that 80% of the carcinogenic nitrosamines and 58% of the carcinogenic hydrocarbons were detected in the assay. These figures were rebutted by Ames and Haroun largely on the basis of inadequacy of carcinogenicity data and were recalculated to suggest that 88% of the carcinogenic polycyclic hydrocarbons were mutagens. Several potent carcinogens in both chemical groups were nonmutagenic in the test (i.e., false-negatives).

The disagreement involves several points. One point of contention is the quality of the cancer data. Often, the data used are from experiments that do not conform to guidelines, such as those suggested by the International Agency for Research on Cancer.[58] Ames and McCann[6] discussed how the inclusion of questionable carcinogenicity data may influence the interpretation of mutagenicity testing. The accuracy and quantitation of carcinogenicity data are dealt with separately in Chapter 3.

Analysis of chemicals by class influences the extent to which carcinogenicity and Salmonella/microsome test data are related. For example, Rinkus and Legator[102] divided the 465 chemicals in their compendium into 38 classes. Some classes were constructed according to structural similarities, and others, such as the antifolates, were based on functional properties. Classes of carcinogens poorly detected in the Salmonella/microsome test included azonaphthols; carbonyls; phenyls; polychlorinated aromatics, cyclics, and aliphatics; and steroids. Ames and McCann[6] have suggested that the

classification scheme of Rinkus and Legator is poorly constructed and its interpretation therefore misleading. However, they encouraged attempts in general to categorize chemicals and suggested that an optimal scheme would require knowledge of the structure of a carcinogen, the enzyme system needed to transform it, and the structure and action of the proximate active forms.

For strictly qualitative purposes, a short-term test battery of mutagenicity tests offers the best chance of detecting most carcinogens. Campbell[35] suggested that the combination of the Salmonella/microsome test and a mammalian cell transformation test could theoretically detect 99.19% of carcinogens--a much higher detection rate than reported for any combination of tests by de Serres and Ashby.[43]

RECENT COMPARATIVE STUDIES

Several large, recent studies have examined the relationship between mutagenicity and carcinogenicity in a laboratory context. It is important to describe the new studies in detail and then to review earlier experimental data from which they were derived.

Committee 2 of the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) recently published its 3-year analysis of mutagenicity testing as an approach to carcinogenicity testing.[59] This group concluded that "the uncertainty about the mechanisms by which chemical carcinogens induce cancer precludes the precise selection of predictive tests on the basis of similarities of mechanisms between the test system and the mammalian model of carcinogenesis." Committee 2 cautioned that the expansion of a test battery will produce an increase in false-positive results and thus necessitate the comparison of in vitro data with results of more protracted animal tests, which themselves are uncertain in predicting cancer in man. Because of the apparent complexity of carcinogenesis and the disparate modes of metabolic activation used in in vitro mutagenicity tests, the quantitative relationship between the two processes was considered very uncertain.

As a member of the ICPEMC committee, Purchase[97] defined three stages of validation for short-term tests in predicting carcinogenicity. Only one "established" test was identified--the Salmonella/microsome test. Nine "developed" tests were named, and 10 tests were described as "developing." Validation studies have suggested that the ability to predict carcinogenicity will not exceed 90% for any test. Thus, a single mutagenicity test was thought to be inadequate for judging the potential carcinogenicity of a chemical. In another context, the ability of a single short-term mutagenicity test to predict

clearly the activity of a chemical in other biologic systems was likewise questioned by the present Committee.[84] This lack of confidence in a single test result was extended in a quantitative sense. The Committee also concluded that the mutagenic potency of chemicals in one mutagenicity test cannot be directly compared with potency determined in other tests.

Both as an ICPEMC member[78] and independently,[79] Mohn discussed the use of bacterial tests for the ranking of chemical mutagens and the use of the tests as screens for carcinogenicity. Although advocating the ranking of chemicals in a well-calibrated test, he discouraged attempts at quantification, because of the specificities of genetic end points to chemical classes and because ranking chemicals in bacterial strains with altered DNA repair or metabolism may be poor estimators of potency in wild-type organisms. Mohn described many carcinogens that are mutagenic in most test systems and those which react in only a few mutagenicity assays. He also listed a few animal and human carcinogens that appear to possess no mutagenic activity, such as dieldrin, saccharin, benzene, cadmium, carbon tetrachloride, and diethylstilbestrol.

Venitt[117] expressed similar doubts about the use of bacterial mutation as a quantitative indicator of carcinogenicity. Like others, he viewed imperfections in the short-term mutagenicity and long-term carcinogenicity data as barriers to establishing a strong conclusion concerning the correlation. Several years earlier, Bartsch[17] reviewed the ability to predict carcinogenicity from mutagenicity data. He, too, considered mutagenicity tests strictly as qualitative screens for potential carcinogenicity. His demonstration of the variability of metabolic activation systems in several human liver extracts argued for a parallel between the variability in the tests and the heterogeneity of the human population in susceptibility to particular chemical carcinogens. Because of the variability in metabolic activation systems and the heterogeneity in cancer susceptibility, he did not consider it feasible at the time to correlate mutagenic and carcinogenic potencies across species.

Two large experimental efforts have recently sought to validate short-term mutagenicity tests and explore their mutagenicity-carcinogenicity relationship. de Serres and Ashby[43] directed an international study designed to assess the effectiveness of short-term tests in detecting carcinogens and noncarcinogens as defined by their activity in rodent systems. Forty-two chemicals--14 carcinogen-noncarcinogen pairs, 11 carcinogens normally inactive in bacterial mutation assays, and three miscellaneous compounds--were tested by 65 collaborators with 35 assays. The pairs of chemicals usually consisted of a well-established carcinogen and a noncarcinogen of similar chemical structure on which data were less sub-

stantial. No assay gave positive results for all carcinogens and negative results for all noncarcinogens. However, a battery of tests was able to detect all carcinogens and all noncarcinogens. The tests were in five categories: bacterial mutation assays, yeast assays, in vitro mammalian cell assays, cytogenetic assays, and in vivo mammalian assays. The two most serious considerations in deriving test batteries were decreasing false-positive and false-negative results and creating maximal complementarity among tests. For example, most of the carcinogens found to be inactive in the Salmonella/microsome assay were positive in at least one in vitro eukaryotic system.

The detail in this large study makes its total review difficult. However, from the data generated in the non-definitive report, a conclusion was reached concerning mutagenic and carcinogenic potencies; because of the inadequacy of the estimates of carcinogenic potency, any correlation was thought to have little general value. Some contradictions are evident in the preponderance of positive mutagenicity-test results versus negative carcinogenicity data. Of the 17 materials originally classified as noncarcinogens on the basis of negative animal carcinogenicity data, eight were reclassified as "true noncarcinogens" according to more extensive mutagenicity testing. False-positive or false-negative mutagenicity results are relative to their agreement with available animal carcinogenicity data, which appear to have a tendency toward false-negatives.

The second recent collaborative study comparing mutagenicity and carcinogenicity results was sponsored by the United Kingdom Environmental Mutagen Society.[90] It was conducted to supplement several aspects of the de Serres-Ashby international study, especially the development of quantitative data. Three chemicals were examined for mutagenicity and carcinogenicity: benzylchloride (BC), 4-chloromethylbiphenyl (4CMB), and 4-hydroxymethylbiphenyl (4HMB). (A serious problem that developed in the course of the study was the unexpected instability of 4CMB.) Bacterial mutagenicity was determined in 17 laboratories; fungal mutagenicity in seven; mammalian cell mutagenesis, transformation, and DNA repair in 14; cytogenetic analysis in 11; in vivo mutagenicity in 10; and mouse skin tumorigenesis in two. In most, all three chemicals were tested. The analysis of bacterial mutagenicity in Salmonella and *E. coli* indicated rather wide differences among laboratories. All laboratories correctly classified 4HMB as a nonmutagen, and all identified 4CMB as a mutagen. The results with BC were less consistent, because it appeared as a "weak" mutagen that acted at restricted sites.. The study group[118] concluded that, although it is extremely unlikely that any useful relationship could be identified between mutagenic and carcinogenic potencies, the analysis of mutagenic potency among related

chemicals that were tested with the same bacterial strains may provide useful quantitative information. The present Committee reached the same conclusion in its earlier report.[84]

The use of mammalian cells in culture (to study either mutation, transformation, or DNA repair) for 4CMB gave the greatest number of positive results: 17 of 26 tests were positive. Of 16 mammalian tests, only two (both measuring cellular transformation) were positive for 4HMB, and eight tests were positive for BC. The mammalian cell study group[11] could give no quantitative interpretation of the data.

The sex-linked recessive lethal test in Drosophila was positive for 4CMB, BC, and probably 4HMB. All three chemicals gave positive results in the Drosophila somatic cell segregation assay. In contrast, the in vivo mouse mutagenicity assays (micronucleus and dominant-lethal tests) gave no evidence of genotoxicity for any chemical. The mouse skin tumor test (conducted in two laboratories) failed to detect any neoplastic changes for any of the materials.

An important general conclusion that can be drawn from the UK study is that, for the three chemicals tested, it was not possible to correlate the data quantitatively among the mutagenicity tests. Indeed, for the most consistent data (bacterial), the UK group recommended that potency be calculated on the basis of the Salmonella/microsome test--and then only for specific bacterial strains. The carcinogenicity results were considered inadequate for deciding whether the consistent negative results accurately described the activity of the compounds. Therefore, because of a general inconsistency in the mutagenicity results and a lack of confidence in the carcinogenicity data, no correlation could be drawn between mutagenic and carcinogenic activities.

As concluded by the Committee in the summary, the results of these large, recent analyses indicate a firm qualitative relationship between the two end points. Further study is required to establish a quantitative correlation.

EARLIER QUALITATIVE STUDIES

Some perspective on efforts to determine the relationship between carcinogenicity and mutagenicity may be afforded by discussing a set of relevant reports in roughly chronologic order, not only to describe the evolution of an emerging consensus, but also to show how improved data may influence the result.

Coombs *et al.*[41] in 1976 analyzed 54 polycyclic compounds, of which 29 were cyclopenta[a]phenanthrenes, 11 chrysenes, and 14 benz[a]anthracenes. Carcinogenic potency was described as the ratio of the incidence of skin tumors to latent period. Mutagenicity data were from the Salmonella/microsome test and were calculated from the slope of the linear portion of the

dose-response curve. All 37 carcinogens were mutagenic, as were seven of 16 noncarcinogens. Despite the number of false-positive results, the authors were impressed with the qualitative correlation for these compounds. However, they concluded that their study did not show a quantitative relationship and questioned whether one could ever be demonstrated. This conclusion was also reached in a more recent analysis.[52] In 1977, a multi-end-point study[19] similar to that of Coombs et al. was performed with N-hydroxy-2-aminofluorene and other derivatives and the Salmonella/-microsome test. The qualitative correspondence among electrophilicity, mutagenicity, DNA-repair induction, and carcinogenicity (sarcomas following subcutaneous injection) was generally high. 2-N-Myristoyloxy derivatives lacked mutagenic activity, but had the other activities. Quantitatively, all the compounds differed markedly with respect to the four test end points.

Bartsch and colleagues have conducted a series of studies on comparative mutagenicity. In 1980,[18] they compared the carcinogenicity and mutagenicity (in Salmonella and CHO cells) of 180 chemicals. Over 90% of the chemical carcinogens were mutagenic in both tests. However, no quantitative correlation was found between mutagenic and carcinogenic activities of these chemicals. In a second study, Bartsch et al.[20] compared hydrocarbon-derived bay-region dihydrodiols for bacterial mutagenicity, Iball indexes (tumor incidence and latency) for carcinogenicity, and DNA-binding in mouse skin. The mutagenicities of the dihydrodiols and the carcinogenicities of the hydrocarbons from which they were derived were much more closely associated than the mutagenicities of the hydrocarbons themselves and their carcinogenicities. The lack of a quantitative correlation between the mutagenicities of the polycyclic hydrocarbons and their carcinogenicities confirms results with dibenzanthracenes[73] and with cyclopentaphenanthrenes.[41]

In 1979, Glatt et al.[49] examined 43 heterocyclic compounds for a mutagenicity-carcinogenicity relationship. Mutagenicity was determined in the Salmonella/microsome test, and carcinogenicity by subcutaneous injection of the compounds into mice. Of the 18 chemicals that produced tumors, one (a weak carcinogen) was not mutagenic in Salmonella. Of the 25 noncarcinogens, 13 were mutagenic to the same extent as the carcinogens. No quantitative correlation was reported.

In 1978, Purchase and colleagues[100] determined how well six short-term tests detected carcinogenicity of 120 organic chemicals: 58 carcinogens and 62 noncarcinogens from several functional and structural classes, including polycyclics, alkylating agents, and aromatic amines. Compounds that were negative in studies that extended over most of the animals' life span were classified as noncarcinogens. The Salmonella/-

microsome test correctly detected 91% of the carcinogens and 94% of the noncarcinogens. Approximately 94% of each type of compound were accurately detected in the cell-transformation test. No attempt was made to determine potency.

In 1979, Poirier and de Serres[93] described the results of an appraisal of mutagenicity screening tests for carcinogenicity, which the National Cancer Institute (NCI)[94] participated in and which involved the comparison of data from several sources. Approximately 150 chemicals were surveyed, including aromatic amines, polycyclic hydrocarbons, nitrosamines and nitrosamides, alkylating agents, and other classes. Each chemical had been classified as a procarcinogen, ultimate carcinogen, or noncarcinogen before to the beginning of the appraisal. Because between 25 and 40% of noncarcinogens in the NCI group were mutagenic, the collaborative group concluded that the Salmonella/microsome test, or any test, could not be used in isolation. The combination of the Salmonella/microsome test and one using an E. coli pol A-deficient strain detected 92% of the organic carcinogens in the NCI list. About 26% of the noncarcinogens were mutagenic in the two tests. The quality of the mutagenicity and carcinogenicity data was considered questionable, and no quantitative analysis was performed.

Another review[30] at the time surveyed a phylogenetically diverse set of mutagenicity tests for 54 carcinogens and noncarcinogens. Positive results were obtained with 100% (17/17) of the ultimate carcinogens, 96% (27/28) of the procarcinogens, and 67% (6/9) of the noncarcinogens in at least one test. The Salmonella/microsome assay detected 84% (37/44) of the carcinogens, but also found 25% (2/8) of the noncarcinogens to be positive. The Drosophila systems gave positive results in 90% (19/21) of the carcinogens and 50% (3/6) of noncarcinogens. No quantitative evaluation was performed.

Although not strictly a genetic end point, DNA repair has also been used in correlations of carcinogenicity and mutagenicity. Rosenkranz and Poirier[104] compared the DNA-modifying activities (E. coli DNA polymerase deficiency) and mutagenic activities (Salmonella/microsome assay) of 99 chemicals. The compounds were in eight chemical classes; 21 were noncarcinogens, 21 were ultimate carcinogens, 45 were procarcinogens, and the remainder could not be classified accurately. All ultimate carcinogens modified DNA, as assayed by growth inhibition of E. coli pol A^-, and 79% were mutagenic; 52% of the procarcinogens were mutagenic and 67% modified DNA; and 39% of the noncarcinogens were mutagenic and 30% modified DNA. The noncarcinogens tested included aniline, anthracene, diphenylnitrosamine, and 5-bromo-2'-deoxyuridine. The high proportion of false-positive results precluded quantitative analysis.

Brambilla and co-workers[28,88] studied the correlation of DNA damage with mutagenicity and carcinogenicity of N-nitroso compounds. As calculated by regression analysis of $\log_{10}$ values of the two activities, correlation was positive between carcinogenic and DNA-modifying activities, but with low statistical significance ($0.10 < p < 0.15$). DNA damage was assayed by alkaline elution of liver DNA from rats exposed in vivo. The mutagenic potency calculated from Salmonella/-microsome data correlated negatively with carcinogenic potency. Carcinogenic potency was defined by the formula, $100(K_t - K_c)/(\text{millimoles per kilogram per day})$, where K is a measure of transforming potency of the cellular environment, t is the exposure duration, and c is the control rate. For the 16 hydrazine derivatives, there was a positive correlation between DNA damage and carcinogenesis, but no correlation between mutagenic and carcinogenic potencies or between mutagenic and DNA-damaging activities.

In a later study,[96] 218 procarcinogens, ultimate carcinogens, and noncarcinogens were compared for their correlation between bacterial mutagenicity and induction of unscheduled DNA synthesis (UDS) in rat hepatocytes. For 67 compounds, the two systems gave positive results at similar dose-response ranges. However, UDS was far less sensitive to nitrosoureas, amides, and other alkylating agents. Negative results for 88 compounds were registered in both tests; most were noncarcinogens, but about 15-20% were carcinogens. Of the 218 chemicals, 48 were positive only in the Salmonella/-microsome test and eight were positive only in the UDS system.

3

QUANTITATIVE CORRELATION BETWEEN MUTAGENICITY AND CARCINOGENICITY

The previous chapter reported experimental studies and data reviews on the qualitative relationship between mutagenicity and carcinogenicity. Most of the studies failed to address whether a quantitative correlation existed, but those which did found no significant correlation. This chapter deals with attempts to discover quantitative relationships, with particular attention to the quality and precision of the quantitative data and to the statistical relationships that have been deduced.

QUANTITATIVE POTENTIAL OF MUTAGENICITY DATA

Ability to extrapolate from chemical mutagens ranked according to the strength of their mutagenicity in simple test systems to effects in systems more relevant to man, such as mammals, would have great advantages in risk estimation. The development of useful algorithms will depend, in part, on the use of relevant measures of mutagenic potency and on the reliability (i.e., reproducibility) of those measures.

McCann and colleagues[24,56,74,75] have begun a thorough statistical examination of estimates of mutagenic potency, using the Salmonella/microsome test as the initial source of data. Others[68,113] have tried to estimate mutagenic potency from assay data by using the entire dose-response curve. However, because chemical toxicity at high doses is not well understood and mutagenic or toxic mechanisms may differ among different chemicals, Bernstein et al.[24] used an alternative approach in which the initial portion of the dose-response curve was estimated with the assumption that it is linear. This assumption is valid if single-hit kinetics for mutagenesis and negligible low-dose toxicity exist. Two measures of potency, calculated from their estimates of the initial slope and intercept of the dose-response curve, have been compared[56]: the slope of the dose-response curve and the ratio of slope to intercept (an estimate of the spontaneous-

revertant background). Salmonella data from the National Cancer Institute/National Toxicology Program were used to show that the initial slope was an appropriate potency measure for one strain (TA100), whereas for three strains (TA98, TA1537, TA1535) the measure incorporating the spontaneous background described the data.

In addition to the NCI/NTP data, Salmonella data from the published literature, representing about 2,500 experiments,[75] were compiled. The D_{100}, the dose required to increase the number of revertants by 100 over the spontaneous background,[24] was chosen as a measure thought to have a more straightforward biologic interpretation of potency than the slope of induced-mutation frequency. Although there is substantial between-experiment variation in potencies obtained for chemicals under the same conditions, the data still tend to cluster. In view of the millionfold range in potency among chemicals in the test, the clustering phenomenon was seen as a hopeful sign in establishing a definitive potency scheme with Salmonella.

McCann and her colleagues[75] have suggested that a single short-term test is unlikely to be sufficient to predict carcinogenic potency. Even for the Salmonella data alone, it will be important to explore the relevance to carcinogenic potency of such factors as chemical class specificity, species-specific activation, and the effect of induction on S-9 homogenates.

The McCann group has also begun a comparison of the activation potential of rat, mouse, and hamster S-9 liver preparations. Initial results suggest that, because the variance of potencies estimated from replicate experiments under the same conditions is high, relatively large differences (up to a factor of 30 or 50) are required to be detected as significant.

In its original report,[84] the Committee discussed the desirability of performing such quantitative exercises with mutagenicity data from a variety of test systems. The Committee stated: "Extrapolation from experimental organisms to man has considerable justification as a qualitative measure, but quantitative extrapolation is uncertain. There is neither sufficient uniformity among systems nor sufficient basic knowledge for quantitative extrapolation to human mutation. Even closely related species differ substantially in metabolism of mutagens and promutagens and in repair capacity."

The Committee's lack of confidence in quantitative mutagenicity data was not absolute, however. If strict adherence to protocols were maintained, it would be possible to rank mutagenicity data within any single test system. Because the range of activity for most tests encompasses several log units, the Committee proposed to classify chemicals within a test according to a logarithmic scale; each log unit would constitute a category. A mutagen could then be described in a semiquantitative, relative way by listing the categories among different test systems. It is important to reiterate that mutagenic

potency was calculated for individual tests and that calculations for different tests should not be numerically combined. Because any chemical may have different results in different tests, this scheme does not permit anything more than a qualitative comparison of groupings among tests.

A formal description of the Committee's mutagenicity potency scheme follows:

Suppose that the least potent chemical in a given test has a value of X, which is in the range 10^k-10^{k+1} units. Each chemical examined in this test is assigned to one of the following potency groups:

Group 1: potency values between 10^k and 10^{k+1}
Group 2: potency values between 10^{k+1} and 10^{k+2}
Group 3: potency values between 10^{k+2} and 10^{k+3}, and so forth

until the highest observed value is reached.

The Committee applied this scheme to data from two large sources: a study of 300 chemicals with the Salmonella/microsome test[73] and the GENE-TOX literature review on mutagenesis in V79 Chinese hamster cells.[27] Both sets of data spanned eight log units. Most mutagens were moderately active in both tests. For the 23 chemicals that were tested in both systems, there was a rough linear correlation. This is an encouraging result, but must be further developed.

As part of a program on comparative chemical mutagenicity, Clive[38] performed an exhaustive analysis of mutagenic potency. Clive used data that had been gathered in the comparative-mutagenicity program and arranged them logarithmically within one test. His rationale for the arrangement was similar to that of the Committee--the large variation of a chemical in the lowest effective concentration that is mutagenic in different test systems. He constructed a mutagenic scale that was normalized to this concentration for each system.

Clive's geometric categorization was within five levels for each test on the scale of the particular test; the width of each category was:

$$\rho = \sqrt[5]{\text{HMD}/\text{LMD}},$$

where HMD and LMD are the highest and lowest mutagenic doses, respectively. Each of the five risk segments is bounded by the expressions: LMD; ρ(LMD); ρ^2(LMD); ρ^3(LMD); ρ^4(LMD); and ρ^5(LMD) = HMD.

For each chemical, a mean risk factor was calculated over all mutagenicity test systems to represent the overall activity of a given chemical. The Committee rejected this extension of relative categorization on the grounds that the use of an average value obscures the great variation in data among tests

and removes the ability to analyze data separately. Also, because the Committee considered strictly mutagenic end points, it did not include Clive's emphasis on in vivo mammalian data on chromosomal aberration in its scheme. Another factor in the Clive evaluation was the consideration of carcinogenicity data, most of which were obtained from IARC.[58] Clive included oncogenicity data in the average risk correlations.

In conclusion, both this Committee and Clive constructed systems determined by a semiquantitative ranking of chemicals. Both tested their systems with experimental data. It is difficult to know whether these rank correlations can be extended to the in vivo systems.

Zimmermann[120] summarized the general problem of extrapolation among systems as one of metabolism--the metabolism of xenobiotic chemicals and the metabolism of DNA. The metabolism of compounds involves the competing processes of activation and detoxification. Physiologic complexities in an organism mask or create mutagenic activities of chemicals in ways that may be peculiar to the organism. The primary target of a mutagen may not always be DNA, but may be a molecular target, such as an enzyme of DNA metabolism, spindle-fiber apparatus, or even a membrane. Because of the influence of these complications in calculating genetic risk, Zimmermann concluded that the mutagenicity of a given chemical is not an intrinsic property of the material, but only a potential that is expressed in combination with biologic characteristics.

QUANTITATIVE POTENTIAL OF CARCINOGENICITY DATA

In animal cancer tests, whole mammals, usually rodents, are used as surrogates for man. Because the biologic end point--cancer--is the same, extrapolation to human risk can be used and adjusted for differences in life span, physiology, susceptibility, and other factors. It has become a regulatory principle that, without human data, only data from animal bioassays are acceptable as definitive evidence of carcinogenicity.

However, cancer bioassays are not simple feeding experiments, and problems of interpretation can arise in bioassays. For example, because the number of animals in an experiment is limited, the highest tolerated doses are often chosen so that the probability of inducing tumors will be as high as possible; but this increases the difficulty of extrapolating to the risk associated with low doses and interpreting the influence of high doses on metabolism of the test chemical, premature mortality, and fitness of the animals. To ensure that experiments follow proper protocols, the National Cancer Institute and the International Agency for Research on Cancer have prepared guidelines for conducting and interpreting carcinogenicity bioassays.

There are some indications that, even among the high standards set by the two agencies cited above, data are not necessarily accurate. For example, Science has reported flaws in experimental design and laboratory performance in the NCI bioassay program, and the results of animal experiments are often controversial.[109]

Qualitatively, the correlation of bioassay data between rodent species appears strong. Purchase[98] analyzed rat and mouse data for 250 chemicals from experiments conducted before 1979. Of these compounds, 44% were carcinogenic and 38% were noncarcinogenic in both rats and mice. Some 64% of the chemicals produced cancer at the same site in both species. Purchase suggested that a chemical positive in one species has about an 85% chance of being positive in a second species.

Several evaluations of cancer data for quantitative potency are underway. Perhaps the most ambitious effort is that of Ames and colleagues.[2,5,50,55] The purposes of their program are to search the literature for acceptable data for making potency estimates and developing a proper data base, to calculate a measure of potency, and to analyze sources of variation.

The Ames group defined as a potency index the tumorigenic dose (TD_{50})--the daily dose rate required to decrease by half the probability of an animal's remaining tumor-free at the end of a standard lifetime test (104 weeks for rats and mice). This index is computed so as to account for spontaneous tumor incidence in control animals and for intercurrent mortality. The data for the index must meet the following criteria: (1) exposure must occur chronically over at least half the animals' normal life span, (2) the route of exposure must be analogous to that of an important human exposure (i.e., diet, gavage, water-drinking, or inhalation), (3) exposure must be to the whole body rather than specific sites, and (4) appropriate controls must be concurrently included in the experiment.

Although Ames reported that over 1,200 experiments with 250 chemicals met these criteria and their results were entered into his data base, only a brief preliminary analysis has been published.[2] In this analysis, a potency range of a factor of 10^7 was observed. TD_{50} values for 18 chemicals that defined this range were depicted. The range in carcinogenic potency that Ames described is very similar to that noted by Fishbein[47] a few years ago.

A more formal statistical evaluation of TD_{50} and a detailed numerical defense will soon be published.[50,107] The proposed TD_{50} index of carcinogenic potency constitutes a refinement of previous attempts, in that the incidences of spontaneous tumors and intercurrent mortality were accounted for.

Crouch and Wilson[42] have formulated an estimate of carcinogenic potency. At low doses, the dose-response curve is linear and defined by

$$P = \alpha + \beta d \text{ for } \alpha \geq 0,\ \beta \geq 0,$$

where P is the lifetime probability of an animal's getting cancer, α is the spontaneous cancer incidence, d is the dose of carcinogen, and β is the potency of the carcinogen [kg(d/mg)]. At high doses, the dose-response curve is

$$P = 1 - (1 - \alpha)\exp \beta d/(1 - \alpha) \text{ for } d << (1 - \alpha)/\beta.$$

Approximately 90 studies from the NCI Carcinogenesis Bioassay Reports were analyzed, and the carcinogenic potencies of chemicals were compared among both sexes of two rodent species.

For many NCI data, a linear correlation deviating by about a factor of 10 was found between the sexes of the two species. The interspecies correlation of potencies was also good (Osborne Mendel rat versus B6C3F1 mouse; Fischer 344 rat versus B6C3F1 mouse). The authors argued that experimental carcinogenesis in one species can be used to estimate potency in another species by determining an "interspecies relative sensitivity factor."

Crouch and Wilson also attempted to correlate the potencies of chemicals between human and experimental cancers. Cancer incidence in man was given by

$$dN/dt = \lambda t^K,$$

where dN/dt is the cancer incidence, t is the elapsed time, and K is a function of the cancer site. Data from several sources were used, including a National Research Council report[85] and Meselson and Russell.[77] Although the data did not correlate well, an interspecies sensitivity ratio between man:mouse and man:rat was calculated to be about 5:1.

The model of Crouch and Wilson must be subjected to more data. The results of many more carcinogenicity bioassays in which interspecies comparisons are possible should be examined, because the results of interspecies carcinogenicity testing sometimes fail to agree, let alone to correlate numerically (e.g., Ames and McCann[6] and Squire[111]). Also, human carcinogenicity data are probably insufficient for accurate calculations of any human sensitivity factors.

Other biologic criteria for modifying carcinogenic potency estimates have been suggested. Squire[111] considered a number of species, and number of sites affected, latency periods, dose-response relationship, and severity of the induced lesions. Thus, according to Squire, the most potent carcinogens induce primarily malignant tumors, at multiple sites, in a short period, at low doses, and in both sexes of several species. Although not explicitly designed to assess independently carcinogenic potency, the IARC criteria for evaluating

carcinogenicity in experimental animals[58] have been used in part by Squire and others to formulate carcinogenic-potency schemes. IARC classifies animal carcinogenicity data as "sufficient, limited, or inadequate." Sufficient evidence indicates an increased incidence of malignant tumors in multiple species, in multiple experiments (preferably with different routes of administration or doses), or to an unusual degree with regard to incidence, site, or type of tumor or age of onset. Limited evidence, which suggests a carcinogenic effect, is limited in that the studies involve a single species, strain, or experiment; inadequate dosage, exposure, duration, or followup period was used, there was poor survival, or too few animals were used; or the neoplasms often occur spontaneously or had been difficult to classify as malignant by histologic criteria. Inadequate evidence was defined as having major qualitative or quantitative limitations that prohibit finding of a carcinogenic effect or as yielding, within limits of the test, a conclusion that the chemical is not carcinogenic.

The value of carcinogenic potency relative to other factors used to assess the carcinogenicity of a chemical may be exemplified by the carcinogenicity ranking scheme of Squire.[112] Six factors are used to rank animal carcinogens, and five are given equal importance. The five categories are potency (according to dose-response data), the number of species affected, types of neoplasms, spontaneous tumor incidence in the control group, and malignancy of the neoplasm. Given slightly more weight (a maximum of 25% of the highest possible total score) are positive results in a battery of genotoxicity tests.

Squire was cautious on the exclusive use of mathematical models to describe biologic events, particularly carcinogenesis. He stated that, "for the same animal data, different models may predict levels of risk that vary widely, indicating the potential error involved in estimating carcinogenic potency or human cancer risks by such methods." To illustrate this point, Squire referred to the controversy surrounding the testing of saccharin.[83]

This warning about treating in a too nearly mathematical way biologic phenomena whose mechanisms are incompletely understood underscores an uncertainty about calculations of carcinogenic potency. As a concept, potency may be the key component in assessing risk; in practice, uncertainties about experiments and their general applicability to biologic facets of carcinogenesis suggest that potency calculations should be only a part of an overall cancer risk estimation.

DIRECT QUANTITATIVE CORRELATION OF MUTAGENIC AND CARCINOGENIC POTENCIES

Short-term tests for mutagenicity were developed to produce an experimental surrogate for animal cancer bioassays. An early study by Meselson and Russell[77] reported a high positive correlation between mutagenic and carcinogenic potencies for a limited number of chemicals, but these preliminary findings have not been sufficiently supported by later data.

The results of Meselson and Russell were based on 14 chemicals that had been tested by McCann et al.[73] for mutagenicity and examined by IARC for carcinogenicity in 1972-1974. Carcinogenic potency was defined as a TD_{50} in rodent bioassays, and mutagenic potency as the reciprocal of the number of micrograms of a chemical that produced 100 revertants in the Salmonella/microsome test. The range of mutagenic and carcinogenic activities for the 14 chemicals was a factor of 10^5. Meselson and Russell found a high correlation, except for several nitroso compounds.

Ashby and Styles[13,14] described the potential implications of the above findings as momentous--and then discussed the difficulties of obtaining a definitive result. Like Squire in his carcinogen ranking scheme,[112] they pointed to other biologic factors--such as metabolic activation, absorption, and chemical half-life--as equal in importance to potency (expressed as a dose-response relationship) in assessing carcinogenic risk. Although Ashby and Styles supported the qualitative value of in vitro short-term testing, they cautioned against making quantitative correlations. They discussed the enzymatic variability of S9 microsomal preparations, which may be the major source of variability among laboratories in results of the Salmonella/microsome test. Differences in carcinogenic potency between animal species were also discussed, especially in regard to the general lack of concordance in S9 enzymatic activity and whole-animal tumor susceptibility.

Ames and Hooper[4] responded that linear correlation between mutagenic and carcinogenic potencies may depend on the particular chemical and its interaction with a particular organism. They acknowledged the exploratory nature of Meselson and Russell's findings. Most important, they agreed that, because the Salmonella/microsome test was designed for maximal sensitivity, varying the test parameters will change the apparent potency; this was suggested as one reason not to expect the correlation to be precise or to hold for every chemical class.

The work of Meselson and Russell is not the only one to assert a quantitative relationship between mutagenicity and carcinogenicity. Jones et al.[60] studied 26 nitrosamines in a

V79 Chinese hamster cell system cocultivated with primary rat hepatocytes. For the index used and the materials tested, a linear relationship between mutagenicity and carcinogenicity was established ($p \leq 0.0001$). The authors suggested that the V79 cell system could complement the Salmonella system.

Reporting on another rodent cell culture system, the L5178Y/TK$^{+/-}$ mouse lymphoma assay, Clive et al.[39] found an approximately linear relationship between oncogenic and mutagenic potencies for 25 chemicals over a potency range of a factor of 10^5. They found that the 100-fold variation in activity for both end points was a consequence more of dosage differences among chemicals than of biologic effects. However, these findings were tempered with the caution that the perceived correlations were between biologically active doses and that the true correlation may be related to cytotoxicity, rather than genotoxicity. Yet, the authors concluded that these correlations serve as a rough estimate of carcinogenic potency.

A parenthetical observation may help to explain some of the differences seen when using Salmonella is used, rather than mammalian cells in culture. Scribner et al.[108] have shown that, if bacterial data are adjusted for a molecular size or partition factor for a given chemical with carcinogenic activity, the correlation approaches that found with the V79 Chinese hamster system. This, of course, leads to the speculation that differences in membrane permeability or target access may explain the poor correlation between bacterial and mammalian cells when a broad range of compounds is considered.

Recent potency studies of other classes of compounds have been conducted with the Salmonella/microsome test. Studying 4-dimethylaminobenzene derivatives, Ashby et al.[12] found no quantitative correlation. Parodi et al.[89] compared the correlation of DNA binding and DNA fragmentation with carcinogenicity and mutagenicity for 21 compounds. From the limited data, a statistical analysis indicated a correlation between DNA binding and carcinogenicity and a weaker correlation between bacterial mutagenicity and carcinogenicity. Hoel et al.[53] have analyzed mathematically the relationship between DNA adduct formation and carcinogenesis. Tumor response could be linearly correlated with the concentration of DNA adducts in the target organs, and the kinetic processes in DNA adduct formation by carcinogens are implicated in the nonlinearities in the dose-response curve for tumor frequency when they occur.

Perhaps the most definitive statement on the quantitative relationship between mutagenicity and carcinogenicity is the recent review of Bartsch, Tomatis, and Malaveille[21] on the basis of IARC carcinogenicity reports. Their first conclusion was a confirmation of the qualitative association between the two phenomena. Their examination concentrated on the 30 chemicals on which human carcinogenicity data are most compelling.

For 130 chemicals on which evidence of experimental carcinogenicity is "sufficient," the qualitative association is equally strong. Four reasons were given for concluding that data are insufficient to establish whether there is a quantitative correlation. First, a universally accepted index of carcinogenic potency has not yet been defined. TD_{50} has been proposed by Ames and colleagues as a standard index and was used in the Meselson and Russell[77] correlative study, but the scientific community has not yet accepted this definition. Second, few correlation studies have been made, and major debate and contradiction remain unresolved. Third, carcinogenicity indexes for experimental animals are rare and do not cover a representative number of chemical classes. Fourth, in regard to man, epidemiologic studies do not include precise dose-response data.

4

SUMMARY AND CONCLUSIONS

The Committee's second assignment was to determine the feasibility of studying the quantitative value of short-term mutagenicity tests for prediction of carcinogenicity. Having reviewed this topic, the Committee does not believe that the National Research Council should conduct a more detailed study now.

There is no doubt that short-term mutagenicity tests, such as the Ames Salmonella/microsome test, have been successful in identifying carcinogens. A substance that is found to be mutagenic in such a test is clearly a possible human carcinogen. This is attested by the very high fraction of known carcinogens that are positive in short-term mutagenicity tests and by the large number of mutagens that were first detected by short-term tests and later found to be positive in animal cancer tests. The difficulty lies in the question of whether such tests can be used for quantitative prediction of human carcinogenicity. The Committee believes that such tests may well have quantitative utility, but that it would be premature to make conclusive statements now. Rather than calling for the judgment of a National Research Council study, the situation calls for more research and analysis of data.

The relation between an initiating event, which may well be mutation, and the eventual development of a cancer is complex and is thought to involve multiple steps. Thus, the prediction of carcinogenicity is more complex than the prediction of DNA damage itself. The activation systems used in short-term mutagenicity tests may not parallel those involved in human carcinogenesis, particularly those related to promotion and proliferation. Furthermore, some carcinogens are not detected in short-term mutagenicity tests. Until these discrepancies are resolved and more data have been analyzed, a National Research Council study will not be justified. This is not to say that we must wait for certainty; that would be the path to inaction. But we believe that studies now in progress may well provide much better information.

Large studies have been started by Ames *et al.*[50] and McCann *et al.*[56,74] and by GENE-TOX. All involve analysis of large bodies of data, and a part-time National Research Council group would not have the facilities for such analysis. We believe that, if a National Research Council assessment is needed, the proper time to undertake it would be when the large analyses now going on are completed.

In conclusion, the Committee on Chemical Environmental Mutagens thinks that it is not now feasible for the National Research Council to study the use of short-term mutagenicity tests for quantitative prediction of human carcinogenicity. Whereas the data are sufficient to establish a firm qualitative association, current models would require additional verification and study to make them sufficient for quantitative prediction. We recommend that the outcome of the several large projects that are in progress be awaited before consideration of a National Research Council study.

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